

VERIFICATION OF TRANSLATION

Kazunori Hashimoto

of 6th Fl., Kantetsu Tsukuba-Science-City Bldg. 1-1-1, Oroshi-machi,
Tsuchiura, Ibaraki, JAPAN

declare as follows:

1. That I am well acquainted with both the English and Japanese languages,
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Kazunori Hashimoto

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Kazunori Hashimoto

Patent Attorney

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【Inventor】

【Address】 c/o CHUGAI RESEARCH INSTITUTE FOR MOLECULAR
MEDICINE, INC. 153-2, Nagai, Niihari-mura, Niihari-gun, IBARAKI

【Name】 Jun-ichi NEZU

【Inventor】

【Address】 c/o CHUGAI RESEARCH INSTITUTE FOR MOLECULAR
MEDICINE, INC. 153-2, Nagai, Niihari-mura, Niihari-gun, IBARAKI

【Name】 Asuka OKU

【Applicant】

【Identification Number】 596102791

【Name or Appellation】 CHUGAI RESEARCH INSTITUTE FOR
MOLECULAR MEDICINE, INC.

【Representative】 Tsutomu Kawaguchi

【Attorney】

【Identification Number】 100102978

【Patent Attorney】

【Name or Appellation】 Hatsushi Shimizu

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[List of Attached Documents]

[Name of Document]	Specification	1
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[Document Name] Specification

[Title of the Invention] Transporter Genes

[Claims]

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[Claim 1] A protein having an activity to transport an organic cation, comprising an amino acid sequence set forth in SEQ ID NO:1 or said amino acid sequence in which one or more amino acids are substituted, deleted, or added.

10 [Claim 2] A protein having an activity to transport an organic cation, encoded by a DNA hybridizing to a DNA comprising a nucleotide sequence set forth in SEQ ID NO: 2.

[Claim 3] A protein having an activity to transport an organic cation, comprising an amino acid sequence set forth in SEQ ID NO:3 or said amino acid sequence in which one or more amino acids are substituted, deleted, or added.

15 [Claim 4] A protein encoded by a DNA hybridizing to a DNA comprising nucleotide sequence set forth in SEQ ID NO: 4 and having an activity to transport an organic cation.

[Claim 5] A DNA encoding the protein according to claim 1 or 3.

20 [Claim 6] A DNA encoding a protein having an activity to transport an organic cation, hybridizing to a DNA comprising a nucleotide sequence set forth in SEQ ID NO: 2.

25 [Claim 7] A DNA encoding a protein having an activity to transport an organic cation, hybridizing to a DNA comprising a nucleotide sequence set forth in SEQ ID NO:4.

[Claim 8] A vector comprising the DNA according to any one of claims 5 to 7.

[Claim 9] A transformant expressively carrying the DNA according to any one of claims 5 to 7.

30 [Claim 10] A method for producing the protein according to any one of claims 1 to 4, the method comprising culturing the transformant according to claim 9.

[Claim 11] An antibody that binds to the protein comprising an amino acid sequence set forth in SEQ ID NO:1.

35 [Claim 12] An antibody that binds to the protein comprising an amino acid sequence set forth in SEQ ID NO:3.

[Detailed Description of the Invention]

[0001]

[Field of the Invention]

The present invention relates to transporters, proteins
5 involved in transport of substances from the outside to the inside
of cells or vice versa.

[0002]

[Prior Art]

10 Recently, the involvement of various transporters localized
on the plasmamembrane in the uptake system for nutrients and
endogenous substances into cells and their transport mechanisms
have been clarified (Tsuji, A. and Tamai, I., Pharm. Res., 13,
963-977, 1996). These transporters recognize the structures of
15 substances to be transported to selectively transport specific
substances. Transporters that recognize the relatively wide range
of structures may possibly recognize foreign substances such as
drugs by mistake, and actively take in them into cells. It is
believed that drugs permeate through the plasmamembrane
fundamentally by simple diffusion depending on their
20 physicochemical properties such as molecular size, hydrophobicity,
and hydrogen-binding capacity. Particularly, in the case of ionic
drugs, only molecules in the non-dissociated form can permeate
through the plasmamembrane according to the pH partition hypothesis.
However, it has become evident that a number of drugs penetrate
25 through the cell membrane by a specific mechanism other than simple
diffusion, that is, an active transport mediated by transporters,
in organs that require efficient exchange of intracellular and
extracellular substances, including small intestine, uriniferous
tubule, placenta, epithelial cells of choroid plexus, hepatocytes,
30 and blood-brain barrier (Tamai, I. and Tsuji, A., Pharmacia, 31,
493-497, 1995; Saito, H. and Inui, K., Igaku no Ayumi, 179, 393-397,
1996; Tamai, I., Yakubutsu Dotai (Pharmacokinetics), 11, 642-650,
1996). For example, it is known that although oral β -lactam
antibiotics of the non-esterified type are amphoteric or negatively
35 charged in physiological pHs and sparingly soluble in fat, they
are readily absorbed through the intestine. The transport study

using the isolated membrane-vesicles system demonstrated that an H⁺-driven peptide transporter localized on the brush-border is involved in the absorption process of these drugs (Okano, T. et al., J. Biol. Chem., 261, 14130-14134, 1986). Although the specificity of a peptide transport system in terms of the peptide size is so strict as to recognize di- or tri-peptides but not tetrapeptides or larger peptides, it has a rather broad substrate specificity to recognize peptides comprising non-natural amino acids. The peptide transporter mediates transport of β -lactam antibiotics mistakenly due to its broad substrate specificity. This property has been unexpectedly utilized in the clinical field (Tsuji, A., American Chemical Society (eds. Taylor, M. D., Amidon, G. L.), Washington, D. C., 101-134, 1995). Furthermore, a possibility that a transporter is also involved in permeation of substances with a high hydrophobicity such as fatty acids through the plasmamembrane has been reported (Schaffer, J. and Lodish, H., Cell, 79, 427-436, 1994).

[0003]

Since various transporters are supposed to be distributed in organs and cells based on the physiological roles of the organs and cells, their distribution and functions may be specific to organs. Therefore, transporters are expected to be used to impart an organ specificity to pharmacokinetics. In other words, an organ-specific drug delivery system (DDS) can be constructed utilizing transporters. If drug absorption solely relied on simple diffusion is improved by elevating its hydrophobicity, an effect of the drug obtained in the initial transport in the liver can be enhanced and the drug can non-specifically translocate into any organ. In addition, it would also be possible to increase the drug absorption independently of its fat-solubility by designing the drug utilizing the substrate specificity of transporters (Hayashi, K. et al., Drug Delivery System, 11, 205-213, 1996). For this purpose, it is necessary to identify various transporters at the molecular level and analyze their properties in detail. However, their molecular level identification are greatly behind studies on their membrane physiology because they

are difficult to handle biochemically and require complicated processes in their functional assays.

[0004]

Recently, cDNAs of several transporters have been cloned by the expression cloning method using *Xenopus* oocytes, a foreign gene expression system, and structural homology among them has been revealed (Fei, Y.-J. et al., *Nature*, 368, 563-566, 1994). For example, Koepsell et al. cloned an organic cation transporter, OCT1, which is assumed to be localized on a basement membrane, using the expression cloning method in 1994 (Grundemann, D. et al., *Nature*, 372, 549-552, 1994). Subsequently, OCT2 was identified by homology cloning based on the sequence of OCT1 (Okuda, M. et al., *Biochem. Biophys. Res. Commun.*, 224, 500-507, 1996). OCT1 and OCT2 show homology as high as 67% to each other, but they differ in their distribution at uriniferous tubules, and OCT2 is believed to be mostly localized at luminal side (Grundemann, D. et al., *J. Biol. Chem.*, 272, 10408-10413, 1997). Both of them are intensely expressed in the kidney, but differ in the organ distribution; OCT1 is also expressed in the liver, colon, and small intestine, while OCT2 expression is specific to the kidney. It has been found that both NKT identified through screening kidney-specific genes (Lopez-Nieto, C.E. et al., *J. Biol. Chem.*, 272, 6471-6478, 1997.) and NLT cloned as an antigen against an antibody that recognizes a membrane protein of hepatic sinusoid (Simonson, G. D. et al., *J. Cell Sci.*, 107, 1065-1072, 1994.) show high homology with OCT1 and OCT2, and therefore, they might constitute one family (OCT family). Besides these, genes derived from *Drosophila* and *C. elegans* which show the same extent of homology are stored in the database (GenBank accession No. Y12400 and Z83228, respectively), and they are thought to be also included in this family. Thus, only a few reports on identification of transporters at the molecular level, including the reports, are available, and there would be many unknown transporters that may be clinically useful.

[0005]

[Problems to be Solved by the Invention]

An object of this invention is to provide a novel transporter.

[0006]

[Means to Solve the Problems]

The present inventors have screened a fetal gene library
5 constructed using the subtractive method by random sequencing based
on a working hypothesis that fetal genes include those which are
involved in various disorders including cancer and are specifically
or intensely expressed in fetal tissues. The inventors discovered
an unknown gene showing a significant homology with those for organic
10 cation transporter family proteins recent-cloned. As a result
of cloning the gene and analyzing its structure, the inventors
found that the gene encodes a novel protein which in overall shows
high homology with organic cation transporter family proteins.
Furthermore, the inventors studied the transporter activity of
15 a protein encoded by the isolated gene and found that the protein,
in fact, functioned as a transporter for various organic cations.

[0007]

This invention relates to a novel transporter, and more
specifically to:

20 (1) a protein having an activity to transport an organic
cation, comprising an amino acid sequence set forth in SEQ ID NO:1,
or a protein comprising said amino acid sequence in which one or
more amino acid residues are substituted, deleted, or added, and
having an activity to transport an organic cation;

25 (2) a protein having an activity to transport an organic
cation, encoded by a DNA hybridizing to a DNA comprising a nucleotide
sequence set forth in SEQ ID NO: 2 and having an activity to transport
an organic cation;

30 (3) a protein having an activity to transport an organic
cation, comprising an amino acid sequence set forth in SEQ ID NO:3,
or a protein comprising said amino acid sequence in which one or
more amino acid residues are substituted, deleted, or added, and
having an activity to transport an organic cation;

35 (4) a protein encoded by a DNA hybridizing to a DNA comprising
nucleotide sequence set forth in SEQ ID NO: 4 and having an activity
to transport an organic cation;

- (5) a DNA encoding the protein according to (1) or (3);
(6) a DNA encoding a protein having an activity to transport an organic cation, hybridizing to a DNA comprising a nucleotide sequence set forth in SEQ ID NO: 2 and encoding the protein having an activity to transport an organic cation;
(7) a DNA encoding a protein having an activity to transport an organic cation, hybridizing to a DNA comprising a nucleotide sequence set forth in SEQ ID NO:4 and encoding the protein having an activity to transport an organic cation;
(8) a vector comprising the DNA according to any one of (5) to (7);
(9) a transformant expressively carrying the DNA according to any one of (5) to (7);
(10) a method for producing the protein according to any one of (1) to (4), the method comprising culturing the transformant according to (9);
(11) an antibody that binds to the protein comprising an amino acid sequence set forth in SEQ ID NO:1; and
(12) an antibody that binds to the protein comprising an amino acid sequence set forth in SEQ ID NO:3.

[0008]

[Mode for Carrying Out the Invention]

This invention relates to a novel transporter protein. Nucleotide sequences of cDNAs of novel human transporters isolated by the present inventors are shown in SEQ ID NO: 2 and SEQ ID NO: 4, respectively. Amino acid sequences of proteins encoded by these cDNAs are shown in SEQ ID NO: 1 and SEQ ID NO: 3, respectively. Amino acid sequences of these two proteins included in the transporter proteins of this invention showed such a high overall homology as about 72%, and both of them retained the following consensus sequence which is conserved in various types of transporters including the glucose transporter: [Leu, Ile, Val, Met, Ser, Thr, Ala, Gly]-[Leu, Ile, Val, Met, Phe, Ser, Ala, Gly]-Xaa<2>-[Leu, Ile, Val, Met, Ser, Ala]-[Asp, Glu]-Xaa-[Leu, Ile, Val, Met, Phe, Tyr, Trp, Ala]-Gly-Arg-[Arg, Lys]-Xaa<4-6>-[Gly, Ser, Thr, Ala] (Maiden, M. C. et al., Nature,

325, 641-643, 1987). In fact, proteins set forth in SEQ ID NO:1 have an activity to transport various organic cations.

[0009]

Transporter proteins of this invention also include those
5 having the additional activity to transport substances other than organic cations as far as they retain the organic cation transport activity. Organic cations include, for example, TEA, carnitine, quinidine, and pyrilamine. Transporter proteins of this invention include those having the activity to transport organic cations
10 not only from the outside to the inside of cells but also from the inside to the outside of cells.

[0010]

Transporter proteins of this invention can be prepared as recombinant proteins using recombination techniques or natural
15 proteins. Recombinant proteins can be prepared, for example, as described below, by culturing cells transformed with DNA encoding proteins of this invention. Natural proteins can be isolated from the kidney and cancer cell strains such as Hela S3, which highly express the proteins of this invention, by the method well known
20 to those skilled in the art, for example, affinity chromatography using an antibody of this invention described below. The antibody may be either polyclonal or monoclonal. A polyclonal antibody can be prepared by purifying serum obtained from, for example, a small animal such as a rabbit immunized with proteins of this
5 invention by known methods, for example, ammonium sulfate precipitation, protein A or protein G column, DEAE-ion exchange column chromatography, affinity column chromatography coupled with the protein of this invention, etc. A monoclonal antibody can be prepared by immunizing a small animal such as a mouse with the
protein of this invention, excising the spleen from the mouse, grinding the tissue into cells, fusing them with mouse myeloma cells using a fusing agent such as polyethyleneglycol, and selecting
a clone that produces an antibody to proteins of this invention out of fused cells (hybridomas) thus produced. Then, hybridomas thus selected are transplanted into the abdominal cavity of a mouse, and the ascites is collected from the mouse. A monoclonal antibody

thus obtained can be purified by, for example, ammonium sulfate precipitation, protein A or protein G column, DEAE-ion exchange column chromatography, affinity column chromatography coupled with the protein of this invention, etc. When the antibody thus obtained is administered to human subjects, a humanized antibody or a human antibody is advantageously used to reduce the immunogenicity. An antibody can be humanized by, for example, the CDR grafting method comprising cloning an antibody gene from monoclonal antibody-producing cells and grafting the epitope portion thereof into an existing human antibody. A human antibody can be prepared by the usual method for preparing a monoclonal antibody except for immunizing a mouse whose immune system is replaced with the human's.

[0011]

It is also possible for those skilled in the art to prepare proteins having functions substantially equivalent to the transporter proteins by appropriately modifying amino acid residues of the proteins set forth in SEQ ID NOS: 1 or 3 by, for example, substitution, using well known methods. Mutation of amino acids of the proteins can occur also spontaneously. Such mutant proteins which are obtained by altering the amino acid sequence of the transporter proteins set forth in SEQ ID NOS: 1 or 3 of this invention by substitution, deletion, or addition of amino acid residues, and have an activity to transport organic cations are also included in the proteins of this invention. Methods well known to those skilled in the art for altering amino acids include, for example, the site-specific mutagenesis system by PCR (GIBCO-BRL, Gaithersburg, Maryland), site-specific mutagenesis by oligonucleotide (Kramer, W. and Fritz, H. J. (1987) Methods in Enzymol., 154: 350-367), Kunkel's method (Methods Enzymol., 85, 2763-2766 (1988)), etc. The number of amino acids that can be substituted is usually 10 amino acid residues or less, preferably 6 or less, and more preferably 3 or less. The site of substitution, deletion, or addition of amino acid residues is not particularly limited as far as the activity of proteins of this invention is retained. It is possible to detect the transporter activity of

proteins, for example, by the method described below in Example 6.

[0012]

It is routine for those skilled in the art to obtain proteins which their function is substantially equivalent to those of the proteins of this invention by isolating and using DNAs highly homologous to the DNA sequences set forth in SEQ ID NOS: 2 or 4 or portions thereof using hybridization techniques (Sambrook, J. et al., Molecular Cloning 2nd ed., 9.47-9.58, Cold Spring Harbor Lab. press, 1989), etc. Therefore, those skilled in the art can prepare proteins which are encoded by a DNA hybridizing to a DNA set forth in SEQ ID NOS: 2 or 4 and have an activity to transport organic cations. These proteins are also included in proteins of this invention. DNAs that hybridize to the DNAs encoding the proteins of this invention can be isolated from other organisms, for example, mice, rats, rabbits, cattle, etc. Especially, tissues such as the kidney are suitable as sources of such DNAs. These DNAs which were isolated using hybridization techniques and encode the proteins having substantially equivalent function to the proteins set forth in SEQ ID NOS: 1 or 3 usually have a high homology with the DNAs set forth in SEQ ID NOS: 2 or 4. "High homology" means at least 70% or more, preferably at least 80% or more, and more preferably at least 90% or more of sequence identity with DNAs set forth in SEQ ID NOS: 2 or 4.

[0013]

One example of hybridization conditions for isolating such DNAs is as follows. That is, after the pre-hybridization at 55°C for 30 min or more in the "ExpressHyb Hybridization Solution" (CLONTECH), a labeled probe is added, and hybridization is performed by heating the reaction mixture at 37°C to 55°C for 1 h or more. Then, the reaction product is successively washed in 2 x SSC and 0.1% SDS three times at room temperature for 20 min, and then in 1 x SSC and 0.1% SDS once at 37°C for 20 min. More preferable conditions are as follows. After the pre-hybridization at 60°C for 30 min or more in the "ExpressHyb Hybridization Solution" (CLONTECH), a labeled probe is added, and hybridization is performed

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by heating the reaction mixture at 60°C for 1 h or more. Then, the reaction product is successively washed in 2 x SSC and 0.1% SDS three times at room temperature for 20 min, and then in 1 x SSC and 0.1% SDS twice at 50°C for 20 min. Still more preferable conditions are as follows. After pre-hybridization at 68°C for 30 min or more in the "ExpressHyb Hybridization Solution" (CLONTECH), a labeled probe is added, and hybridization is performed by heating the reaction mixture at 68°C for 1 h or more. Then, the reaction product is successively washed in 2 x SSC and 0.1% SDS three times at room temperature for 20 min, and then in 0.1 x SSC and 0.1% SDS twice at 50°C for 20 min.

[0014]

The present invention also relates to DNAs encoding the above-described transporter proteins of this invention. DNAs of this invention may be cDNA, genomic DNAs, and synthetic DNAs. The DNAs of the present invention can be used for producing proteins of this invention as recombinant proteins, as well as for isolating proteins which their function is substantially equivalent to those of proteins set forth in SEQ ID NOs:1 or 3. That is, it is possible to prepare proteins of this invention as recombinant proteins by inserting DNAs encoding proteins of this invention (e.g. DNAs set forth in SEQ ID NOs: 2 or 4) into an appropriate expression vector, culturing transformants obtained by transfecting suitable cells with the vector, and purifying the proteins thus expressed. Cells to be used for producing recombinant proteins include, for example, mammalian cells such as COS cells, CHO cells, NIH3T3 cells, etc., insect cells such as Sf9 cells, yeast cells, *E. coli*, and so on. Vectors used for the intracellular expression of recombinant proteins vary depending on host cells, including, for example, pcDNA3 (Invitrogen), pEF-BOS (Nucleic Acids Res., 1990, 18(7), p5322), etc. for mammalian cells, "BAC-to-BAC baculovirus expression system" (GIBCO BRL), etc. for insect cells, "Pichia Expression Kit" (Invitrogen), etc. for yeast cells, pGEX-5X-1 (Pharmacia), "QIAexpress system" (Qiagen), etc. for *E. coli*. Host cells can be transformed with vectors, for example, by the calcium phosphate method, the DEAE-dextran method, the method using

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cationic liposome DOTAP (Boehringer Mannheim), the electroporation method, the calcium chloride method, etc. Recombinant proteins can be purified from recombinants thus obtained using standard methods, for example, as described in "The Qiaexpressionist Handbook, Qiagen, Hilden, Germany."

[0015]

The DNAs of this invention can be used in gene therapy for disorders caused by abnormalities in the activity and expression of the proteins of this invention. In this case, the DNAs of this invention are inserted into an adenovirus vector (e.g. pAdexLcw), a retrovirus vector (e.g. pZIPneo), etc., and administered into the body by either *ex vivo* method or *in vivo* method. Gene therapy can also be performed by administering a synthetic antisense DNA to the body either directly or after inserting into the above-described vector.

[0016]

The transporter proteins of this invention can be used to control internal absorption and dynamics of drugs. Based on the results of detailed analysis of the substrate specificity of transporter proteins of this invention, drugs can be designed so as to be transported by these transporters and absorbability of the drugs mediated by these transporter proteins can be improved. Conventional modifications to enhance hydrophobicity are no longer necessary for drugs so designed, which enables speedily and efficiently developing water-soluble drugs that are easy to handle. The drugs thus developed are thought to be absorbed principally depending on the internal distribution pattern of transporter proteins of this invention, and an organ-specific delivery of the drugs thus becomes possible. Especially, if the transporter proteins of this invention are distributed in the target organ of a drug, an ideal drug delivery system (DDS) can be developed. If a drug is to be absorbed mediated by not the transporter proteins of this invention but other transporters, the drug can be designed so as to be specific to other transporter proteins by designing it considering the substrate specificity of the transporter proteins of this invention. Since the transporter proteins of

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this invention are present in the kidney, it is possible to reduce the nephrotoxicity produced by a drug by designing the drug so that it can be readily excreted by the transporter proteins of this invention.

5 [0017]

Another possible application of this invention is to develop a drug targeting the transporter proteins of this invention. The transporters play important roles in the absorption mechanism of nutrients and drugs, or the excretion mechanism of drugs and internal metabolites. Thus, damage or abnormal elevation of the transporter's functions may cause some disorders. It is considered to be efficacious against such disorders to administer a drug containing a compound that inhibits or enhances functions of the transporter proteins of this invention, or regulates the expression level of the transporter gene of this invention and the amount of the transporter proteins. Also, the above-mentioned gene therapy will be efficacious.

[0018]

The transporter proteins of this invention are expressed in a variety of cancer cell strains, which suggests that the proteins may transport drugs into tumor cells. If this is the case, it is possible to develop carcinostatics that will be readily absorbed mediated by the transporter proteins of this invention. On the contrary, mechanisms to transport and excrete substances by the transporter proteins of this invention may function to excrete carcinostatics in tumor cells so that the cells acquire resistance to drugs. If the transporter proteins of this invention are involved in a mechanism of tumor cells to acquire drug resistance, a carcinostatic effect can be enhanced by using inhibitors of the transporter proteins of this invention.

[0019]

The present invention is described below in more detail with reference to examples, but is not construed being limited thereto.

[0020]

35 [Examples]

Example 1 Construction of a subtraction library

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A subtraction library was constructed using the PCR-Select™ cDNA Subtraction Kit (CLONTECH) principally according to the method of Luda Diatchenko (Diatchenko, L. et al., Proc. Natl. Acad. Sci. USA, 93, 6025-6030, 1996).

5 [0021]

First, double-stranded cDNAs were synthesized from poly(A)⁺ RNAs derived from human fetal liver and adult liver by the standard method using MMLV reverse transcriptase. These cDNAs were blunt-ended with T4 DNA polymerase and cleaved with RsaI. A part of the cDNAs derived from fetal liver (tester) was divided in two portions, and they were separately ligated to two different adapters, adapter 1 and adapter 2, respectively (Table 1). A 120-fold excess of cDNA derived from adult liver (driver) was added to each of the above-described tester samples. The mixture was heat-denatured and subjected to the primary hybridization at 68°C for 8 h. After these two reaction mixtures from the primary hybridization were mixed together without heat-denaturation, an excessive amount of the heat-denatured driver was further added thereto, and the mixture was subjected to the secondary hybridization at 68°C for about 16 h. The resulting reaction solution was diluted with a dilution buffer and incubated at 75°C for 7 min. After the shorter strands of adapters were removed, the reaction solution was used as a template for PCR. PCR using primers 1 (5'-CTAATACGACTCACTATAGGGC-3', SEQ ID NO: 5) and 2 (5'-TGTAGCGTGAAGACGACAGAA-3', SEQ ID NO: 6) corresponding to the adapters selectively amplified only cDNAs having different adapters at their both ends (subtracted cDNAs) (suppression PCR). PCR was carried out using a portion of the resulting cDNA as a template, and nested PCR primers 1 (5'-TCGAGCGGCCGCCCCGGGCAGGT-3', SEQ ID NO: 7) and 2 (5'-AGGGCGTGTTGCGGAGGGCGGT-3', SEQ ID NO: 8), which are further inwardly located from the PCR primers 1 and 2, to obtain products with further elevated selectivity. PCR products thus obtained were purified using the QIAquick PCR Purification kit (QIAGEN), and cloned into the pT7Blue-T vector (Novagen) by the TA cloning method to construct a subtraction library.

[0022]

[Table 1]

Adapter 1	5'-CTAATACGACTCACTATAGGGCTCGAGCGGCCGCGCCGGGCAGGT-3' 3'-GGCCCGTCCA-5'
Adapter 2	5'-TGTAGCGTGAAGACGACAGAAAGGGCGTGGTGC GGAGGGCGGT-3' 3'-GCCTCCCGCCA-5'

Example 2 cDNA cloning

To analyze fetal genes, the subtraction library derived from the fetal liver was screened by random sequencing. Homology search (Blastx) of Expressed Sequence Tags (ESTs) thus obtained found a clone, fls631(292 bp) encoding amino acid sequence having significant homology with the known organic cation transporters, OCT1 (Grundemann, D. et al., Nature, 372, 549-552, 1994), OCT2 (Okuda, M. et al., Biochem. Biophys. Res. Commun., 224, 500-507, 1996), and their family proteins(OCT family protein). Since the sequence of this clone was novel and assumed to be a fragment derived from a new transporter gene, cDNA comprising the whole open reading frame (ORF) of this gene was cloned.

[0023]

The human fetal liver 5'-stretch cDNA library (CLONTECH) was screened using the original fls631 clone obtained from the subtraction library derived from fetal liver as a probe. An insert of the original fls631 clone was amplified by PCR using M13 P4-22 and M13 P5-22, and labeled with [α -³²P]dCTP by the random primer method using the Ready-to Go DNA labeling beads (Pharmacia) to serve as a probe. Hybridization was carried out at 68°C in the ExpressHyb Hybridization Solution (CLONTECH) according to the method recommended by the manufacturer. Final washing was performed at 50°C in 0.1 x SSC and 0.1% SDS. Screening about 5 x 10⁵ phage clones finally isolated seven positive clones. cDNA inserts of these clones were amplified by PCR using vector primers designed based on a sequence of the λ gt10 vector (GT10 S1 5'-CTTTTGAGCAAGTTCAGCCT-3', SEQ ID NO: 9, and GT10 A1 5'-AGAGGTGGCTTATGAGTATTTCTT-3', SEQ ID NO: 10), or primers designed based on the decoded cDNA sequences. The PCR products thus obtained were directly sequenced to determine the nucleotide sequences. Some regions that were difficult to be amplified were

subjected to PCR using 7-deaza dGTP as a substrate base (McConlogue, L. et al., Nucleic Acids Res., 16, 9869, 1988).

[0024]

After screening about 5×10^5 phage clones, finally seven positive clones were isolated. Sequencing of cDNA inserts of these clones revealed that the fls631 gene contains an ORF encoding a protein consisting of 551 amino acid residues (putative molecular weight of about 62,000). Data base search using this whole amino acid sequence confirmed that it has a significant homology not only with the region corresponding to the original fls631 clone but also in overall with known OCT family proteins. Figure 1 shows a comparison between amino acid sequences of fls631 and OCT family proteins (rat OCT1, rat OCT2, mouse NKT(Lopez-Nieto, C. E. et al., J. Biol. Chem., 272, 6471-6478, 1997.), and rat NLT(Simonson, G.D., et al., J. Cell Sci., 107, 1065-1072, 1994.)). The fls631 showed high homology at the amino acid level to these family proteins, being 32%, 33%, 28%, and 28%, respectively. Hydrophobicity profile of this sequence obtained by Kyte & Doolittle's calculating formula (Kyte, J. and Doolittle, R. F., J. Mol. Biol., 157, 105-132, 1982) very closely resembled those of OCT family, indicating that the sequence has eleven to twelve putative transmembrane hydrophobic regions (Fig. 2). This sequence contained one consensus sequence of sugar transporter, ([Leu, Ile, Val, Met, Ser, Thr, Ala, Gly]-[Leu, Ile, Val, Met, Phe, Ser, Ala, Gly]-Xaa<2>-[Leu, Ile, Val, Met, Ser, Ala]-[Asp, Glu]-Xaa-[Leu, Ile, Val, Met, Phe, Tyr, Trp, Ala]-Gly-Arg-[Arg, Lys]-Xaa<4-6>-[Gly, Ser, Thr, Ala]), (160 to 175). This consensus sequence is present in the glucose transporters GLUT1 to GLUT7 in mammalian cells, and also present in various types of transporters other than glucose transporters (Maiden, M. C. et al., Nature, 325, 641-643, 1987). Furthermore, putative N-linked glycosylation sequences (N-X-[ST]) were found in the amino acid sequence of fls631 at four sites (57 to 59, 64 to 66, 91 to 93, and 304 to 306). Though possibility of modification by N-linked sugar chain has been suggested for the OCT family, it has not been biochemically proved, and its biological significance has not been clarified yet. And there were five

putative protein kinase C phosphorylation sites ([ST]-X-[RK]) (164 to 166, 225 to 227, 280 to 282, 286 to 288, and 530 to 532). Among them, particularly the 286-288 site among them was conserved at a common position in all OCN family proteins. In addition, the consensus sequence ([Ala, Gly]-Xaa(4)-Gly-Lys-[Ser, Thr]) of the ATP/GTP binding site is also found. This consensus sequence of the ATP/GTP binding site is also present in the ATP binding protein or GTP binding protein, such as kinases and ras family proteins, and that ATP or GTP binds to this site (Walker, J. E. et al., EMBO J., 1, 945-951, 1982). This sequence is present in the so-called ATP Binding Cassette (ABC) type transporter, and involved in the substance transport using the energy generated by hydrolysis of ATP (Higgins, C. F. et al., J. Bioenerg. Biomembr., 22, 571-592, 1990; Urbatsch, I. L. et al., J. Biol. Chem., 270, 26956-26961, 1995). Presence of this consensus sequence indicates that fls631 protein may be an ATP or GTP-dependent transporter.

[0025]

Nucleotide sequencing was performed by the cycle-sequencing method with a plasmid DNA prepared by the alkaline-SDS method or a PCR product obtained by colony PCR, etc. as a template using the ABI PRISM™ Dye Terminator Cycle Sequencing Ready Reaction Kit With AmplyTaq DNA Polymerase, FS, followed by decoding with the ABI 377 DNA Sequencer (Perkin Elmer). Colony PCR was carried out by directly suspending a colony of a recombinant in a PCR reaction solution containing vector primers M13 P4-22 (5'-CCAGGGTTTCCAGTCACGAC-3', SEQ ID NO: 11) and M13 P5-22 (5'-TCACACAGGAAACAGCTATGAC-3', SEQ ID NO: 12). After the completion of PCR, a DNA insert thus amplified was separated from unreacted primers and nucleotides by gel filtration, etc. to serve as a template for sequencing.

[0026]

Example 3 Northern analysis

Distribution of fls631 in tissues was investigated by Northern analysis (Fig. 3). A 3'-end fragment of fls631 (the latter half from around the base 1,100) was labelled with [α -³²P]dCTP by the random primer method using the Ready-to Go DNA labeling beads

(Pharmacia) to serve as a probe. Hybridization was performed using the Multiple Tissue Northern (MTN) Blot - Human, Human III, Human IV, Human Fetal II, and Human Cell lines (CLONTECH) at 68°C in the ExpressHyb Hybridization Solution (CLONTECH) according to the method recommended by the manufacturer. Final washing was performed at 50°C in 0.1 x SSC and 0.1% SDS. As a result, RNA of about 2.5 kb was strongly expressed in the fetal liver and adult-derived tissues such as the kidney, bone marrow, and trachea. Besides those tissues, the RNA band was also weakly detected in the fetal kidney and lung, and adult tissues including skeletal muscle, lung, placenta, prostate, spleen, and spinal cord. The RNA expression was also detected in tumor cell lines such as HeLa S3, K562, SW480, and A549, and especially, its very intense expression was observed in HeLa S3.

[0027]

Example 4 Cloning of 631RT cDNA

Data base search using the entire nucleotide sequence of "fls631" detected very similar sequences thereto in several parts of the nucleotide sequence of P1 phage clones (P1 H24 clones, GenBank accession No. L43407, L43408, L46907, L81773, and L43409) derived from q regions of human chromosome 5 reported at Human Genome Center. The parts having similarity with the nucleotide sequence of fls631 but being obviously different from it are included. The similar sequence are separated by the sequences regarding as intron. The sequence obtained by connecting these similar parts with each other with reference to the sequence of OCTN1 has a high homology over a wide range with fls631 (Fig 4), indicating the presence of fls631 homologues like OCT1 and OCT2 relationship. Therefore, in order to perform cDNA cloning of this homologous gene, 631R S4 primer (5'-GTGCTGTTGGGCTCCTTCATTTCA-3', SEQ ID NO: 13) and 631RA1 primer (5'-AGCTGCATGAAGAGAAGGACACTG-3', SEQ ID NO: 14) were prepared based on sequences of these P1 phage clones. PCR was performed using a set of these primers and cDNA synthesized from poly(A)⁺ RNA derived from the human adult kidney (CLONTECH) as a template, under the following conditions: 1 cycle of 94°C for 3 min; 35 cycles of 94°C for 30 s, 58°C for 1 min, and 72°C for 2 min,; and 1 cycle

of 72°C for 10 min, resulting in amplification of about 900 bp fragment. This fragment was subcloned into the pT7Blue-T vector (Novagen) by the TA cloning method to determine its nucleotide sequence, which clearly showed a very high overall homology with fls631. Therefore, this gene was designated as 631RT (fls 631 Related Transporter), and longer cDNAs were cloned.

[0028]

Renewably, the 631R S6 primer (5'-AGCATCCTGTCTCCCTACTTCGTT-3', SEQ ID NO: 15) was prepared. Cloning was performed by the Rapid Amplification of cDNA Ends (RACE) method (Chenchik, A., Moqadam, F., and Siebert, P. (1995), CLONTECHniques X, 5-8). PCR was performed using this primer and the Marathon-Ready™ cDNA derived from the human adult kidney (CLONTECH) as a template under the following conditions: 94°C for 2 min; 35 cycles of 94°C for 30 s, 60°C for 1 min, and 72°C for 3 min,; and 72°C for 10 min, resulting in amplification of about 1.7 kbp cDNA fragment of the 3'-end. This fragment was subcloned into the pT7Blue-T vector by the TA cloning method. The partly nucleotide sequence determination of the clone revealed that it was a fragment of the 3'-end of 631RT. The sequence obtained by these methods was shown in SEQ ID NO: 3.

[0029]

It became evident that the sequence contains an open reading frame (ORF) encoding a protein consisting of 460 amino acid residues. Fig. 5 compares amino acid sequences of the ORF and fls631. Both showed overall amino acid homology as high as about 72%. In addition, one consensus sequence of sugar transporter was present in the amino acid sequence of 631RT like fls631. These facts indicated that 631RT can be a novel transporter that is structurally related to fls631. Furthermore, a consensus sequence of the ATP/GTP binding site was also present in the amino acid sequence of 631RT like fls631. The 631RT cDNA comprising a longer region could be cloned using the cDNA already obtained as a probe in the same manner as for fls631 cDNA cloning.

[0030]

Example 5 Northern analysis

Northern analysis was performed using about 900 bp 631RT cDNA as a probe which was obtained by PCR with a set of 631R S4 primer (5'-GTGCTGTTGGGCTCCTTCATTTC-3', SEQ ID NO: 13) and 631RA1 primer (5'-AGCTGCATGAAGAGAAGGACACTG-3', SEQ ID NO: 14) in the same manner as for fls631. The results are shown in Fig. 6. Although the expression pattern of 631RT partly overlapped with that of fls631, 631RT differs from fls631 in that the former was very intensely expressed in the kidney among fetal tissues, while 631RT was strongly expressed also in cancer cell strains such as K-562, HeLa S3, SW480, etc. as well as the kidney, indicating that fls631 and 631RT may be involved in transport of substances such as carcinostatics in these cancer cells.

[0031]

Example 6 Forced expression of fls631 in human fetal kidney cells (HEK293) and its activity determination

Phage DNAs were extracted from positive phage clones obtained by screening the clones by the plaque hybridization method using the QIAGEN Lambda Kit (QIAGEN). After the DNA insert was subcloned into the pUC18 vector, cDNA containing the entire ORF which was cleaved out with SmaI and EcoRI was integrated between the EcoRI site and the blunted HindIII site of an expression vector for mammalian cells, pCDNA3 (Invitrogen), to obtain an expression plasmid DNA, pCDNA3/631-2. Plasmid DNA was prepared by alkaline-SDS method using the QIAGEN PLASMID MAXI Kit (QIAGEN).

[0032]

The human fetal kidney-derived cell strain, HEK 293 cells were transfected with the plasmid pCDNA3/631-2 and pCDNA3 vector containing no insert as a control by the calcium phosphate method. First, the plasmid DNA (10 μ g), a Hepes buffer solution (137 mM NaCl, 5 mM KCl, 0.7 mM Na_2HPO_4 , 6 mM Dextrose, and 21 mM Hepes pH 7.1) (1 ml), and 2M CaCl_2 (62.5 μ l) were combined and allowed to stand at room temperature for 30 min or more to form calcium phosphate coprecipitates. After cells were plated on 10-cm plates at 1.5×10^6 cells per plate and cultured for 24 h, the calcium phosphate coprecipitates were added thereto, and the cells were further cultured for 24 h. Then, plates were washed with phosphate buffered

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saline (PBS), and the cells were further cultured for 24 h after the addition of fresh culture medium.

[0033]

Transport experiment was performed using cells transfected with the plasmid DNA or untreated cells according to the following procedures. Cells were detached from plates using a rubber policeman, suspended in a transport buffer (containing 125 mM NaCl, 4.8 mM KCl, 5.6 mM (+)-glucose, 1.2 mM CaCl₂, 1.2 mM KH₂PO₄, 1.2 mM MgSO₄, and 25 mM Hepes pH 7.4), and pre-incubated for 20 min.

10 An appropriate amount of each labeled substrate ([¹⁴C]TEA (tetraethylammonium) (NEN), [³H]carnitine (L-carnitine hydrochloride) (Amersham), [³H]PCG (benzylpenicillin) (Amersham), [³H]quinidine (ARC), or [³H]pyrilamine (mepyramine) (Amersham)) was then added to the cell suspension, and the resulting mixture
15 was incubated at 37°C for a predetermined period of time. Incubated cells were overlaid on a silicon layer prepared by layering a mixture of silicon oil and liquid paraffin (specific gravity = 1.022) on a 3 M KCl layer, and separated by centrifugation. Radioactivity of cells was measured to determine the into-the-cell transport
20 activity. In this case, 1 x 10⁶ cells were used as one point of cells. HEK 293 cells were cultured in Dulbecco's MEM containing 10% fetal calf serum (FCS) in an atmosphere of 5% carbon dioxide at 37°C.

[0034]

25 First, the transporter capacity was measured in the cells transfected with pCDNA3/fls631-2 and untreated cells using TEA as a substrate (Fig. 7). A reaction time-dependent TEA uptake into the fls631-transfected cells was clearly observed. This uptake was not observed in untreated cells. Next, effects of the addition
30 of unlabeled TEA on the labeled substrate uptake in this system (cold inhibition) were examined (Fig. 8). A decrease in the apparent uptake of the labeled substrate was clearly seen depending on the concentration of cold TEA added. In this experiment, almost no uptake of the substrate into cells was observed in cells
35 transfected with the pCDNA3 vector containing no insert (Mock) used as a control like in untreated cells used, clearly indicating

that this uptake phenomenon is due to the transfection of the cells with fls631. Next, to obtain the K_m (Michaelis constant) value of fls631 to TEA, the uptake of ^{14}C -TEA with various concentrations was measured (Fig. 9). From Lineweaver-Burk reciprocal plot of the net uptake obtained by subtracting the amount of the uptake in Mock cells from that in the human fls631-transfected cells, the K_m value of 0.44 ± 0.04 mM was obtained with the maximal velocity, V_{max} of 6.68 ± 0.34 (nmol/3 min/mg). Next, the transport capacity of OCTN1 for other substrate than TEA was examined (Fig. 10). When the transport capacity was measured using labeled organic cations such as labeled carnitine, quinidine, and pyrilamine, a significant increase in the uptake of these compounds was clearly observed in fls631-transfected cells as compared with Mock cells, clearly indicating that these organic cations can serve as substrates for fls631. However, no significant increase in the uptake of an organic anion, PCG (benzylpenicillin), was observed.

[0035]

[Effect of the Invention]

This invention provides a novel organic cation transporter. Transporter of this invention are useful for developing newly designed drugs that can be transported mediated by these proteins, and pharmaceuticals for disorders caused by functional abnormalities of the proteins.

[Sequence Listing]

SEQ ID NO: 1

LENGTH: 551

TYPE: amino acid

5 TOPOLOGY: linear

MOLECULE TYPE: protein

SEQUENCE

Met Arg Asp Tyr Asp Glu Val Ile Ala
1 5
10 Phe Leu Gly Glu Trp Gly Pro Phe Gln Arg Leu Ile Phe Phe Leu Leu
10 15 20 25
Ser Ala Ser Ile Ile Pro Asn Gly Phe Asn Gly Met Ser Val Val Phe
30 35 40
Leu Ala Gly Thr Pro Glu His Arg Cys Arg Val Pro Asp Ala Ala Asn
15 45 50 55
Leu Ser Ser Ala Trp Arg Asn Asn Ser Val Pro Leu Arg Leu Arg Asp
60 65 70
Gly Arg Glu Val Pro His Ser Cys Ser Arg Tyr Arg Leu Ala Thr Ile
75 80 85
20 Ala Asn Phe Ser Ala Leu Gly Leu Glu Pro Gly Arg Asp Val Asp Leu
90 95 100 105
Gly Gln Leu Glu Gln Glu Ser Cys Leu Asp Gly Trp Glu Phe Ser Gln
110 115 120
Asp Val Tyr Leu Ser Thr Val Val Thr Glu Trp Asn Leu Val Cys Glu
25 125 130 135
Asp Asn Trp Lys Val Pro Leu Thr Thr Ser Leu Phe Phe Val Gly Val
140 145 150
Leu Leu Gly Ser Phe Val Ser Gly Gln Leu Ser Asp Arg Phe Gly Arg
155 160 165
30 Lys Asn Val Leu Phe Ala Thr Met Ala Val Gln Thr Gly Phe Ser Phe
170 175 180 185
Leu Gln Ile Phe Ser Ile Ser Trp Glu Met Phe Thr Val Leu Phe Val
190 195 200
Ile Val Gly Met Gly Gln Ile Ser Asn Tyr Val Val Ala Phe Ile Leu
35 205 210 215
Gly Thr Glu Ile Leu Gly Lys Ser Val Arg Ile Ile Phe Ser Thr Leu

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Met Gln Lys Val Lys Trp Phe Arg Ser Gly Lys Lys Thr Arg Asp Ser
525 530 535
Met Glu Thr Glu Glu Asn Pro Lys Val Leu Ile Thr Ala Phe
5 540 545 550

SEQ ID NO: 2

LENGTH: 2135

TYPE: nucleic acid

10 STRANDEDNESS: double

TOPOLOGY: linear

MOLECULE TYPE: cDNA to mRNA

FEATURE

NAME/KEY: CDS

15 LOCATION: 147..1799

DEFINITION METHOD: E

SEQUENCE

CCCCGGCTTC GCGCCCAAT TTCTAACAGC CTGCCTGTCC CCCGGAACG TTCTAACATC 60
CTTGGGGAGC GCCCCAGCTA CAAGACACTG TCCTGAGAAC GCTGTCATCA CCCGTAGTTG 120
20 CAAGTTTCGG AGCGGCAGTG GGAAGC ATG CGG GAC TAC GAC GAG GTG ATC GCC 173
Met Arg Asp Tyr Asp Glu Val Ile Ala
1 5
TTC CTG GGC GAG TGG GGG CCC TTC CAG CGC CTC ATC TTC TTC CTG CTC 221
Phe Leu Gly Glu Trp Gly Pro Phe Gln Arg Leu Ile Phe Phe Leu Leu
25 10 15 20 25
AGC GCC AGC ATC ATC CCC AAT GGC TTC AAT GGT ATG TCA GTC GTG TTC 269
Ser Ala Ser Ile Ile Pro Asn Gly Phe Asn Gly Met Ser Val Val Phe
30 35 40
CTG GCG GGG ACC CCG GAG CAC CGC TGT CGA GTG CCG GAC GCC GCG AAC 317
30 Leu Ala Gly Thr Pro Glu His Arg Cys Arg Val Pro Asp Ala Ala Asn
45 50 55
CTG AGC AGC GCC TGG CGC AAC AAC AGT GTC CCG CTG CGG CTG CGG GAC 365
Leu Ser Ser Ala Trp Arg Asn Asn Ser Val Pro Leu Arg Leu Arg Asp
60 65 70
35 GGC CGC GAG GTG CCC CAC AGC TGC AGC CGC TAC CGG CTC GCC ACC ATC 413
Gly Arg Glu Val Pro His Ser Cys Ser Arg Tyr Arg Leu Ala Thr Ile

	75	80	85	
	GCC AAC TTC TCG GCG CTC GGG CTG GAG CCG GGG CGC GAC GTG GAC CTG			461
	Ala Asn Phe Ser Ala Leu Gly Leu Glu Pro Gly Arg Asp Val Asp Leu			
	90	95	100	105
5	GGG CAG CTG GAG CAG GAG AGC TGC CTG GAT GGC TGG GAG TTC AGC CAG			509
	Gly Gln Leu Glu Gln Glu Ser Cys Leu Asp Gly Trp Glu Phe Ser Gln			
	110	115	120	
	GAC GTC TAC CTG TCC ACC GTC GTG ACC GAG TGG AAT CTG GTG TGT GAG			557
	Asp Val Tyr Leu Ser Thr Val Val Thr Glu Trp Asn Leu Val Cys Glu			
10	125	130	135	
	GAC AAC TGG AAG GTG CCC CTC ACC ACC TCC CTG TTC TTC GTA GGC GTG			605
	Asp Asn Trp Lys Val Pro Leu Thr Thr Ser Leu Phe Phe Val Gly Val			
	140	145	150	
	CTC CTC GGC TCC TTC GTG TCC GGG CAG CTG TCA GAC AGG TTT GGC AGG			653
15	Leu Leu Gly Ser Phe Val Ser Gly Gln Leu Ser Asp Arg Phe Gly Arg			
	155	160	165	
	AAG AAC GTT CTC TTC GCA ACC ATG GCT GTA CAG ACT GGC TTC AGC TTC			701
	Lys Asn Val Leu Phe Ala Thr Met Ala Val Gln Thr Gly Phe Ser Phe			
	170	175	180	185
20	CTG CAG ATT TTC TCC ATC AGC TGG GAG ATG TTC ACT GTG TTA TTT GTC			749
	Leu Gln Ile Phe Ser Ile Ser Trp Glu Met Phe Thr Val Leu Phe Val			
	190	195	200	
	ATC GTG GGC ATG GGC CAG ATC TCC AAC TAT GTG GTA GCC TTC ATA CTA			797
	Ile Val Gly Met Gly Gln Ile Ser Asn Tyr Val Val Ala Phe Ile Leu			
25	205	210	215	
	GGA ACA GAA ATT CTT GGC AAG TCA GTT CGT ATT ATA TTC TCT ACA TTA			845
	Gly Thr Glu Ile Leu Gly Lys Ser Val Arg Ile Ile Phe Ser Thr Leu			
	220	225	230	
	GGA GTG TGC ACA TTT TTT GCA GTT GGC TAT ATG CTG CTG CCA CTG TTT			893
30	Gly Val Cys Thr Phe Phe Ala Val Gly Tyr Met Leu Leu Pro Leu Phe			
	235	240	245	
	GCT TAC TTC ATC AGA GAC TGG CGG ATG CTG CTG CTG GCG CTG ACG GTG			941
	Ala Tyr Phe Ile Arg Asp Trp Arg Met Leu Leu Leu Ala Leu Thr Val			
	250	255	260	265
35	CCG GGA GTG CTG TGT GTC CCG CTG TGG TGG TTC ATT CCT GAA TCT CCC			989
	Pro Gly Val Leu Cys Val Pro Leu Trp Trp Phe Ile Pro Glu Ser Pro			

270 275 280
 CGA TGG CTG ATA TCC CAG AGA AGA TTT AGA GAG GCT GAA GAT ATC ATC 1037
 Arg Trp Leu Ile Ser Gln Arg Arg Phe Arg Glu Ala Glu Asp Ile Ile
 285 290 295
 5 CAA AAA GCT GCA AAA ATG AAC AAC ACA GCT GTA CCA GCA GTG ATA TTT 1085
 Gln Lys Ala Ala Lys Met Asn Asn Thr Ala Val Pro Ala Val Ile Phe
 300 305 310
 GAT TCT GTG GAG GAG CTA AAT CCC CTG AAG CAG CAG AAA GCT TTC ATT 1133
 Asp Ser Val Glu Glu Leu Asn Pro Leu Lys Gln Gln Lys Ala Phe Ile
 10 315 320 325
 CTG GAC CTG TTC AGG ACT CGG AAT ATT GCC ATA ATG ACC ATT ATG TCT 1181
 Leu Asp Leu Phe Arg Thr Arg Asn Ile Ala Ile Met Thr Ile Met Ser
 330 335 340 345
 TTG CTG CTA TGG ATG CTG ACC TCA GTG GGT TAC TTT GCT CTG TCT CTG 1229
 15 Leu Leu Leu Trp Met Leu Thr Ser Val Gly Tyr Phe Ala Leu Ser Leu
 350 355 360
 GAT GCT CCT AAT TTA CAT GGA GAT GCC TAC CTG AAC TGT TTC CTC TCT 1277
 Asp Ala Pro Asn Leu His Gly Asp Ala Tyr Leu Asn Cys Phe Leu Ser
 365 370 375
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 Ala Leu Ile Glu Ile Pro Ala Tyr Ile Thr Ala Trp Leu Leu Leu Arg
 380 385 390
 ACG CTG CCC AGG CGT TAT ATC ATA GCT GCA GTA CTG TTC TGG GGA GGA 1373
 Thr Leu Pro Arg Arg Tyr Ile Ile Ala Ala Val Leu Phe Trp Gly Gly
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 Gly Val Leu Leu Phe Ile Gln Leu Val Pro Val Asp Tyr Tyr Phe Leu
 410 415 420 425
 TCC ATT GGT CTG GTC ATG CTG GGA AAA TTT GGG ATC ACC TCT GCT TTC 1469
 30 Ser Ile Gly Leu Val Met Leu Gly Lys Phe Gly Ile Thr Ser Ala Phe
 430 435 440
 TCC ATG CTG TAT GTC TTC ACT GCT GAG CTC TAC CCA ACC CTG GTC AGG 1517
 Ser Met Leu Tyr Val Phe Thr Ala Glu Leu Tyr Pro Thr Leu Val Arg
 445 450 455
 35 AAC ATG GCG GTG GGG GTC ACA TCC ACG GCC TCC AGA GTG GGC AGC ATC 1565
 Asn Met Ala Val Gly Val Thr Ser Thr Ala Ser Arg Val Gly Ser Ile

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      460      465      470
ATT GCC CCC TAC TTT GTT TAC CTC GGT GCT TAC AAC AGA ATG CTG CCC      1613
Ile Ala Pro Tyr Phe Val Tyr Leu Gly Ala Tyr Asn Arg Met Leu Pro
      475      480      485
5  TAC ATC GTC ATG GGT AGT CTG ACT GTC CTG ATT GGA ATC TTC ACC CTT      1661
   Tyr Ile Val Met Gly Ser Leu Thr Val Leu Ile Gly Ile Phe Thr Leu
      490      495      500      505
TTT TTC CCT GAA AGT TTG GGA ATG ACT CTT CCA GAA ACC TTA GAG CAG      1709
Phe Phe Pro Glu Ser Leu Gly Met Thr Leu Pro Glu Thr Leu Glu Gln
10      510      515      520
   ATG CAG AAA GTG AAA TGG TTC AGA TCT GGG AAA AAA ACA AGA GAC TCA      1757
   Met Gln Lys Val Lys Trp Phe Arg Ser Gly Lys Lys Thr Arg Asp Ser
      525      530      535
   ATG GAG ACA GAA GAA AAT CCC AAG GTT CTA ATA ACT GCA TTC      1799
15  Met Glu Thr Glu Glu Asn Pro Lys Val Leu Ile Thr Ala Phe
      540      545      550
TGAAAAAATA TCTACCCCAT TTGGTGAAGT GAAAAACAGA AAAATAAGAC CCTGTGGAGA      1859
AATTCGTTGT TCCCACTGAA ATGGACTGAC TGTAACGATT GACACCAAAA TGAACCTTGC      1919
TATCAAGAAA TGCTCGTCAT ACAGTAAACT CTGGATGATT CTTCCAGATA ATGTCCTTGC      1979
20  TTTACAAACC AACCATTCTT AGAGAGTCTC CTTACTCATT AATTCAATGA AATGGATTGG      2039
   TAAGATGTCT TGAAAACATG TTAGTCAAGG ACTGGTAAAA TACATATAAA GATTAACACT      2099
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SEQ ID NO: 3

25 LENGTH: 460

TYPE: amino acid

TOPOLOGY: linear

MOLECULE TYPE: protein

SEQUENCE

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   Cys Leu Asp Gly Trp Glu Phe Ser Gln Asp Val Tyr Leu Ser Thr Ile
      20      25      30
Val Thr Glu Trp Asn Leu Val Cys Glu Asp Asp Trp Lys Ala Pro Leu
35      35      40      45
Thr Ile Ser Leu Phe Phe Val Gly Val Leu Leu Gly Ser Phe Ile Ser

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50 55 60
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 65 70 75
 Met Gly Thr Gln Thr Gly Phe Ser Phe Leu Gln Ile Phe Ser Lys Asn
 5 80 85 90 95
 Phe Glu Met Phe Val Val Leu Phe Val Leu Val Gly Met Gly Gln Ile
 100 105 110
 Ser Asn Tyr Val Ala Ala Phe Val Leu Gly Thr Glu Ile Leu Gly Lys
 115 120 125
 10 Ser Val Arg Ile Ile Phe Ser Thr Leu Gly Val Cys Ile Phe Tyr Ala
 130 135 140
 Phe Gly Tyr Met Val Leu Pro Leu Phe Ala Tyr Phe Ile Arg Asp Trp
 145 150 155
 Arg Met Leu Leu Val Ala Leu Thr Met Pro Gly Val Leu Cys Val Ala
 15 160 165 170 175
 Leu Trp Trp Phe Ile Pro Glu Thr Pro Arg Trp Leu Ile Ser Gln Gly
 180 185 190
 Arg Phe Glu Glu Ala Glu Val Ile Ile Arg Lys Ala Ala Lys Ala Asn
 195 200 205
 20 Gly Val Val Val Pro Ser Thr Ile Phe Asp Pro Ser Glu Leu Gln Asp
 210 215 220
 Leu Ser Ser Lys Lys Gln Gln Ser His Asn Ile Leu Asp Leu Leu Arg
 225 230 235
 Thr Trp Asn Ile Arg Met Val Thr Ile Met Ser Ile Met Leu Trp Met
 25 240 245 250 255
 Thr Leu Ser Val Gly Tyr Phe Gly Leu Ser Leu Asp Thr Pro Asn Leu
 260 265 270
 His Gly Asp Ile Phe Val Asn Cys Phe Leu Ser Ala Met Val Glu Val
 275 280 285
 30 Pro Ala Tyr Val Leu Ala Trp Leu Leu Leu Gln Tyr Leu Pro Arg Arg
 290 295 300
 Tyr Ser Met Ala Thr Ala Leu Phe Leu Gly Gly Ser Val Leu Leu Phe
 305 310 315
 Met Gln Leu Val Pro Val Asp Leu Tyr Tyr Leu Ala Thr Val Leu Val
 35 320 325 330 335
 Met Val Gly Lys Phe Gly Val Thr Ala Ala Phe Ser Met Val Tyr Val

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11
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30
77

	340	345	350	
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	Val Ser Ser Thr Ala Ser Arg Leu Gly Ser Ile Leu Ser Pro Tyr Phe			
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	Val Tyr Leu Gly Ala Tyr Asp Arg Phe Leu Pro Tyr Ile Leu Met Gly			
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	Ser Leu Thr Ile Leu Thr Ala Ile Leu Thr Leu Phe Leu Pro Glu Ser			
	400	405	410	415
10	Phe Gly Thr Pro Leu Pro Asp Thr Ile Asp Gln Met Leu Arg Val Lys			
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	Gly Met Lys His Arg Lys Thr Pro Ser His Thr Arg Met Leu Lys Asp			
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SEQ ID NO: 4

LENGTH: 1555

TYPE: nucleic acid

20 STRANDEDNESS: double

TOPOLOGY: linear

MOLECULE TYPE: cDNA to mRNA

SEQUENCE

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	TGT CTG GAT GGC TGG GAG TTC AGT CAG GAC GTC TAC CTG TCC ACC ATT	95
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	Val Thr Glu Trp Asn Leu Val Cys Glu Asp Asp Trp Lys Ala Pro Leu	
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	ACA ATC TCC TTG TTC TTC GTG GGT GTG CTG TTG GGC TCC TTC ATT TCA	191
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	GGG CAG CTG TCA GAC AGG TTT GGC CGG AAG AAT GTG CTG TTC GTG ACC	239

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Met Gly Thr Gln Thr Gly Phe Ser Phe Leu Gln Ile Phe Ser Lys Asn
5 80 85 90 95
TTT GAG ATG TTT GTC GTG CTG TTT GTC CTT GTA GGC ATG GGC CAG ATC 335
Phe Glu Met Phe Val Val Leu Phe Val Leu Val Gly Met Gly Gln Ile
100 105 110
TCC AAC TAT GTG GCA GCA TTT GTC CTG GGG ACA GAA ATT CTT GGC AAG 383
10 Ser Asn Tyr Val Ala Ala Phe Val Leu Gly Thr Glu Ile Leu Gly Lys
115 120 125
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Ser Val Arg Ile Ile Phe Ser Thr Leu Gly Val Cys Ile Phe Tyr Ala
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Phe Gly Tyr Met Val Leu Pro Leu Phe Ala Tyr Phe Ile Arg Asp Trp
145 150 155
CGG ATG CTG CTG GTG GCG CTG ACG ATG CCG GGG GTG CTG TGC GTG GCA 527
Arg Met Leu Leu Val Ala Leu Thr Met Pro Gly Val Leu Cys Val Ala
20 160 165 170 175
CTC TGG TGG TTC ATC CCT GAG ACC CCC CGA TGG CTC ATC TCT CAG GGA 575
Leu Trp Trp Phe Ile Pro Glu Thr Pro Arg Trp Leu Ile Ser Gln Gly
180 185 190
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25 Arg Phe Glu Glu Ala Glu Val Ile Ile Arg Lys Ala Ala Lys Ala Asn
195 200 205
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Thr Trp Asn Ile Arg Met Val Thr Ile Met Ser Ile Met Leu Trp Met
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	His	Gly	Asp	Ile	Phe	Val	Asn	Cys	Phe	Leu	Ser	Ala	Met	Val	Glu	Val	
5					275					280						285	
	CCA	GCA	TAT	GTG	TTG	GCC	TGG	CTG	CTG	CTG	CAA	TAT	TTG	CCC	CGG	CGC	911
	Pro	Ala	Tyr	Val	Leu	Ala	Trp	Leu	Leu	Leu	Gln	Tyr	Leu	Pro	Arg	Arg	
					290					295						300	
	TAT	TCC	ATG	GCC	ACT	GCC	CTC	TTC	CTG	GGT	GGC	AGT	GTC	CTT	CTC	TTC	959
10	Tyr	Ser	Met	Ala	Thr	Ala	Leu	Phe	Leu	Gly	Gly	Ser	Val	Leu	Leu	Phe	
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	ATG	CAG	CTG	GTA	CCC	GTG	GAC	TTG	TAT	TAT	TTG	GCT	ACA	GTC	CTG	GTG	1007
	Met	Gln	Leu	Val	Pro	Val	Asp	Leu	Tyr	Tyr	Leu	Ala	Thr	Val	Leu	Val	
					320					325						330	
15	ATG	GTG	GGC	AAG	TTT	GGA	GTC	ACG	GCT	GCC	TTT	TCC	ATG	GTC	TAC	GTG	1055
	Met	Val	Gly	Lys	Phe	Gly	Val	Thr	Ala	Ala	Phe	Ser	Met	Val	Tyr	Val	
					340					345						350	
	TAC	ACA	GCC	GAG	CTG	TAT	CCC	ACA	GTG	GTG	AGA	AAC	ATG	GGT	GTG	GGA	1103
	Tyr	Thr	Ala	Glu	Leu	Tyr	Pro	Thr	Val	Val	Arg	Asn	Met	Gly	Val	Gly	
20					355					360						365	
	GTC	AGC	TCC	ACA	GCA	TCC	CGC	CTG	GGC	AGC	ATC	CTG	TCT	CCC	TAC	TTC	1151
	Val	Ser	Ser	Thr	Ala	Ser	Arg	Leu	Gly	Ser	Ile	Leu	Ser	Pro	Tyr	Phe	
					370					375						380	
	GTT	TAC	CTT	GGT	GCC	TAC	GAC	CGC	TTC	CTG	CCC	TAC	ATT	CTC	ATG	GGA	1199
25	Val	Tyr	Leu	Gly	Ala	Tyr	Asp	Arg	Phe	Leu	Pro	Tyr	Ile	Leu	Met	Gly	
					385					390						395	
	AGT	CTG	ACC	ATC	CTG	ACA	GCC	ATC	CTC	ACC	TTG	TTT	CTC	CCA	GAG	AGC	1247
	Ser	Leu	Thr	Ile	Leu	Thr	Ala	Ile	Leu	Thr	Leu	Phe	Leu	Pro	Glu	Ser	
					400					405						410	
30	TTC	GGT	ACC	CCA	CTC	CCA	GAC	ACC	ATT	GAC	CAG	ATG	CTA	AGA	GTC	AAA	1295
	Phe	Gly	Thr	Pro	Leu	Pro	Asp	Thr	Ile	Asp	Gln	Met	Leu	Arg	Val	Lys	
					420					425						430	
	GGA	ATG	AAA	CAC	AGA	AAA	ACT	CCA	AGT	CAC	ACA	AGG	ATG	TTA	AAG	GAT	1343
	Gly	Met	Lys	His	Arg	Lys	Thr	Pro	Ser	His	Thr	Arg	Met	Leu	Lys	Asp	
35					435					440						445	
	GGT	CAA	GAA	AGG	CCC	ACA	ATC	CTT	AAA	AGC	ACA	GCC	TTC	TAACATCGCT			1392

Gly Gln Glu Arg Pro Thr Ile Leu Lys Ser Thr Ala Phe

450

455

460

TCCAGTAAGG GAGAACTGA AGAGGAAAGA CTGTCTTGCC AGAAATGGCC AGCTTGTGCA 1452

GACCGAGTCC TTCAGTGACA AAAGGCCTTT GCTGTTTGTC CTCTTGACCT GTGTCTGACT 1512

5 TGCTCCTGGA TGGGCACCCA CACTCAGAGG CTACATATGG CCC 1555

SEQ ID NO: 5

LENGTH: 22

TYPE: nucleic acid

10 STRANDEDNESS: single

TOPOLOGY: linear

MOLECULE TYPE: other synthetic DNA

SEQUENCE

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15

SEQ ID NO: 6

LENGTH: 21

TYPE: nucleic acid

STRANDEDNESS: single

20 TOPOLOGY: linear

MOLECULE TYPE: other synthetic DNA

SEQUENCE

TGTAGCGTGA AGACGACAGA A 21

25 SEQ ID NO: 7

LENGTH: 22

TYPE: nucleic acid

STRANDEDNESS: single

TOPOLOGY: linear

30 MOLECULE TYPE: other synthetic DNA

SEQUENCE

TCGAGCGGCC GCCCGGGCAG GT 22

SEQ ID NO: 8

35 LENGTH: 22

TYPE: nucleic acid

STRANDEDNESS: single

TOPOLOGY: linear

MOLECULE TYPE: other synthetic DNA

SEQUENCE

5 AGGGCGTGGT GCGGAGGGCG GT

22

SEQ ID NO: 9

LENGTH: 20

TYPE: nucleic acid

10 STRANDEDNESS: single

TOPOLOGY: linear

MOLECULE TYPE: other synthetic DNA

SEQUENCE

CTTTTGAGCA AGTTCAGCCT

20

15

SEQ ID NO: 10

LENGTH: 24

TYPE: nucleic acid

STRANDEDNESS: single

20 TOPOLOGY: linear

MOLECULE TYPE: other synthetic DNA

SEQUENCE

AGAGGTGGCT TATGAGTATT TCTT

24

25 SEQ ID NO: 11

LENGTH: 22

TYPE: nucleic acid

STRANDEDNESS: single

TOPOLOGY: linear

30 MOLECULE TYPE: other synthetic DNA

SEQUENCE

CCAGGGTTTT CCCAGTCACG AC

22

SEQ ID NO: 12

35 LENGTH: 22

TYPE: nucleic acid

STRANDEDNESS: single
TOPOLOGY: linear
MOLECULE TYPE: other synthetic DNA
SEQUENCE

5 TCACACAGGA AACAGCTATG AC 22

SEQ ID NO: 13

LENGTH: 24

TYPE: nucleic acid

10 STRANDEDNESS: single

TOPOLOGY: linear

MOLECULE TYPE: other synthetic DNA

SEQUENCE

GTGCTGTTGG GCTCCTTCAT TTCA 24

15

SEQ ID NO: 14

LENGTH: 24

TYPE: nucleic acid

STRANDEDNESS: single

20 TOPOLOGY: linear

MOLECULE TYPE: other synthetic DNA

SEQUENCE

AGCTGCATGA AGAGAAGGAC ACTG 24

25 SEQ ID NO: 15

LENGTH: 24

TYPE: nucleic acid

STRANDEDNESS: single

TOPOLOGY: linear

30 MOLECULE TYPE: other synthetic DNA

SEQUENCE

AGCATCCTGT CTCCTACTT CGTT 24

[Brief Description of the Drawings]

[Figure 1] Figure 1 represents the comparison of amino acid sequences of OCT family proteins. The initial letters of "h" indicates human-derived, "m" indicates mouse-derived, and "r" indicates rat-derived.

[Figure 2] Figure 2 represents hydrophobicity plots of OCT family proteins according to Kyte & Doolittle's calculating formula with a window of nine amino acid residues.

[Figure 3] Figure 3 represents electrophoretic patterns showing the results of Northern blot analysis of fls631.

[Figure 4] Figure 4 compares the fls631 similar sequence derived from q regions of human chromosome 5 (5q Genome) and fls631 sequence.

[Figure 5] Figure 5 compares the amino acid sequence of fls631 with that of 631RT. Sequences coinciding with the consensus sequences of sugar transporter and the ATP/GTP binding site are indicated by "+" and "*", respectively.

[Figure 6] Figure 6 represents electrophoretic patterns showing the results of Northern blot analysis of 631RT.

[Figure 7] Figure 7 is a graph showing the TEA-absorbing activity of fls631. Clear circles represent untreated cells, and solid circles represent fls631-transfected cells.

[Figure 8] Figure 8 is a graph showing effects of the cold TEA added in the experimental system in Fig. 7. In this graph, solid circles represent fls631-transfected cells, and clear circles represent cells containing the vector with no insert. Clear triangles indicate the net uptake induced by fls631 obtained by subtracting the clear circle values from the corresponding solid circle values.

[Figure 9] Figure 9 is a graph showing TEA concentration-dependency of the TEA-absorbing activity of fls631.

[Figure 10] Figure 10 is a bar graph showing the activity of the fls631-transfected cells to absorb substances other than TEA.

Drawings

【Figure 1】

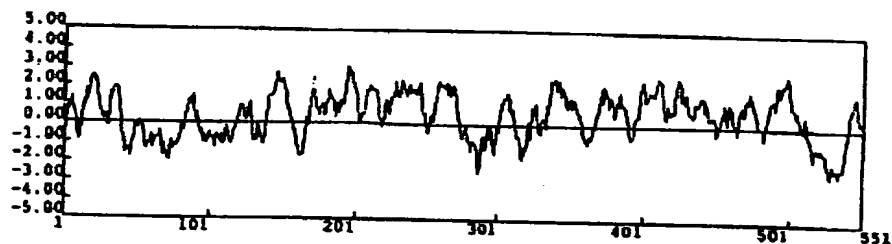
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Reference No. = C2-906

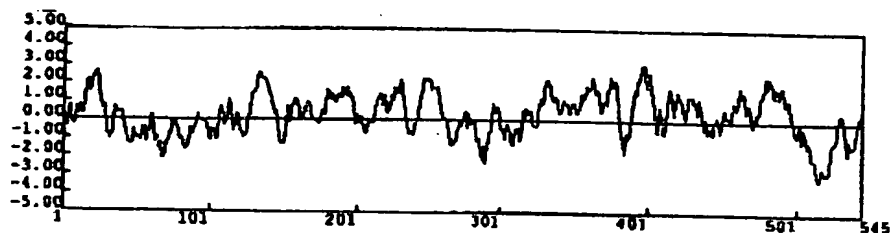
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【Figure 2】

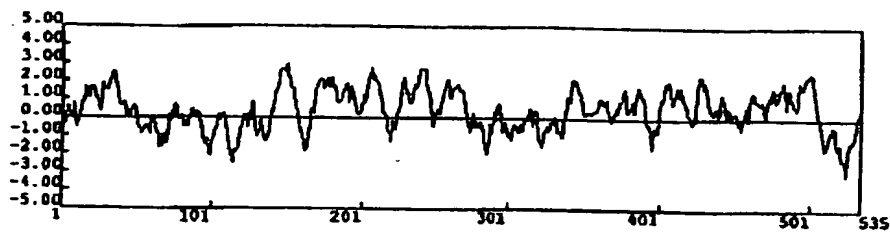
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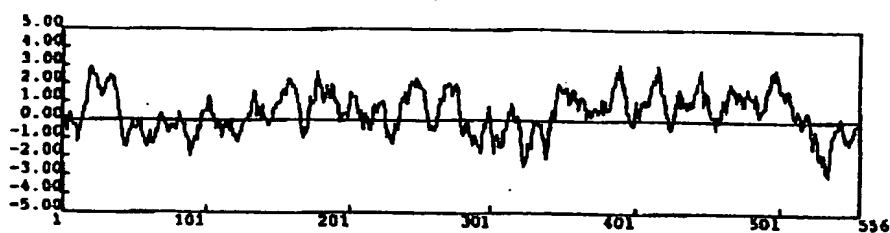
mNKT



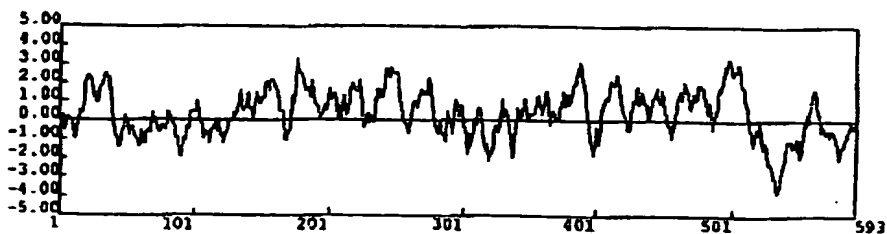
rNLT



rOCT1



rOCT2



Cell lines
derived from human cancer

Melanoma G361
Lung cancer A549
Large intestine adenocarcinoma SW480
Burkitt's lymphoma Raji
Lymphoblastic leukemia MOLT-4
Chronic myelocytic leukemia K-562
Cervical carcinoma HeLa S3
Promyelocytic leukemia HL-60

2.5kb

(Figure 3)

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[illegible]

Reference No. = C2-906

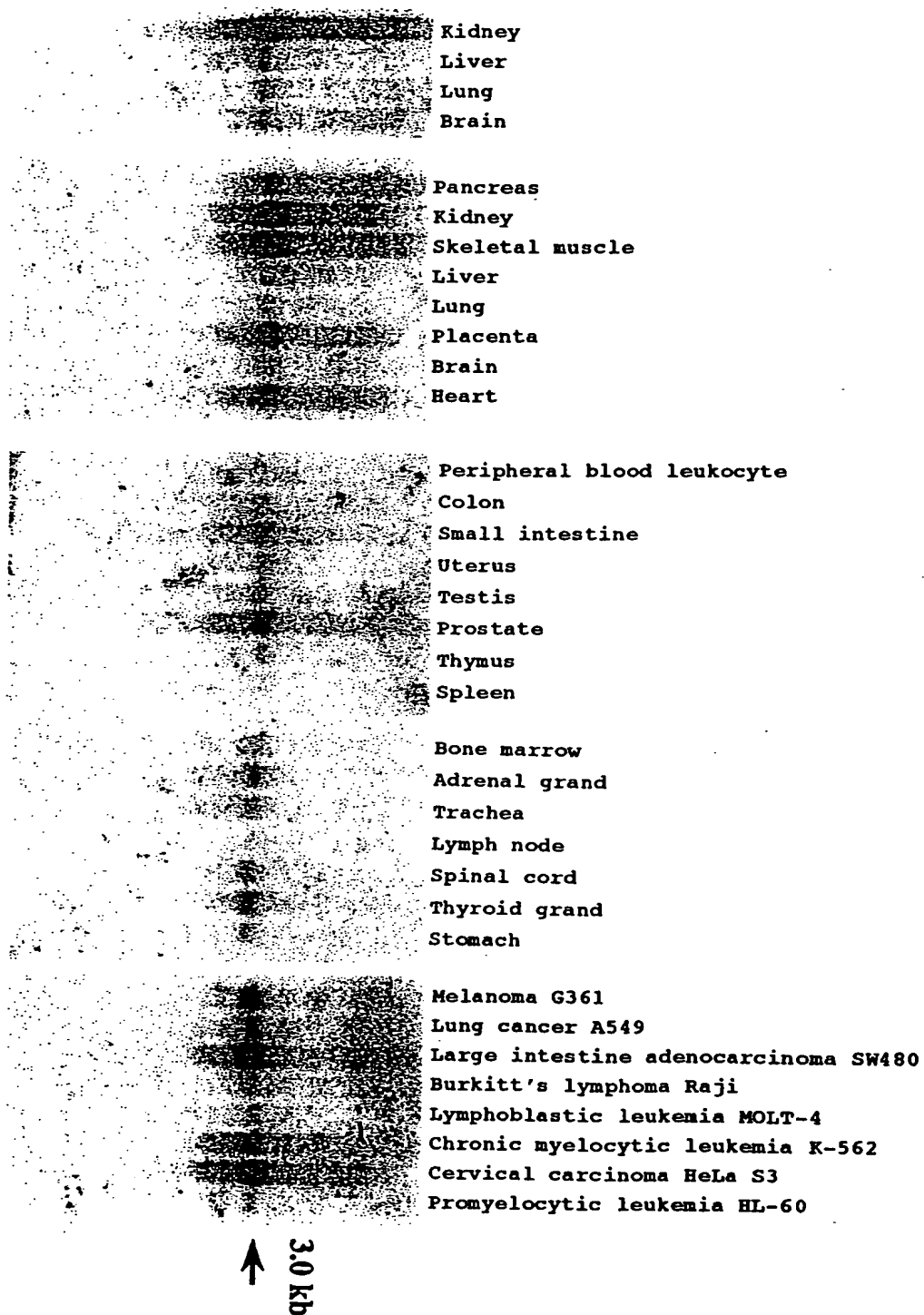
(5/10)

【Figure 5】

	10	20	30	40	50	
f1s631	MRDYDEVIAF	LGEWGPFQRL	IFFLLSASII	PNGFNGMSVV	FLAGTPEHRC	
631RT	-----	-----	-----	-----	-----	
	60	70	80	90	100	
f1s631	RVPDAANLSS	AWRNNSVPLR	LRDGREVPHS	CSRYRLATIA	NFSALGLD	
631RT	-----	-----	-----	-----	-----	
	110	120	130	140	150	
f1s631	RVVETVTS	RVVETVTS	RVVETVTS	RVVETVTS	RVVETVTS	
631RT	RVVETVTS	RVVETVTS	RVVETVTS	RVVETVTS	RVVETVTS	
	160	170	180	190	200	
f1s631	ISWETVTS	ISWETVTS	ISWETVTS	ISWETVTS	ISWETVTS	
631RT	ISWETVTS	ISWETVTS	ISWETVTS	ISWETVTS	ISWETVTS	
	210	220	230	240	250	
f1s631	VEVETVTS	VEVETVTS	VEVETVTS	VEVETVTS	VEVETVTS	
631RT	VEVETVTS	VEVETVTS	VEVETVTS	VEVETVTS	VEVETVTS	
	260	270	280	290	300	
f1s631	REVDVTS	REVDVTS	REVDVTS	REVDVTS	REVDVTS	
631RT	REVDVTS	REVDVTS	REVDVTS	REVDVTS	REVDVTS	
	310	318	328	338	348	
f1s631	AIMETVTS	AIMETVTS	AIMETVTS	AIMETVTS	AIMETVTS	
631RT	AIMETVTS	AIMETVTS	AIMETVTS	AIMETVTS	AIMETVTS	
	358	368	378	388	398	
f1s631	RVVETVTS	RVVETVTS	RVVETVTS	RVVETVTS	RVVETVTS	
631RT	RVVETVTS	RVVETVTS	RVVETVTS	RVVETVTS	RVVETVTS	
	408	418	428	438	448	
f1s631	SAESLYFT	SAESLYFT	SAESLYFT	SAESLYFT	SAESLYFT	
631RT	SAESLYFT	SAESLYFT	SAESLYFT	SAESLYFT	SAESLYFT	
	458	468	478	488	498	
f1s631	RVVETVTS	RVVETVTS	RVVETVTS	RVVETVTS	RVVETVTS	
631RT	RVVETVTS	RVVETVTS	RVVETVTS	RVVETVTS	RVVETVTS	
	508	518	528	535	545	551
f1s631	MLKDGQRET	MLKDGQRET	MLKDGQRET	MLKDGQRET	MLKDGQRET	MLKDGQRET
631RT	MLKDGQRET	MLKDGQRET	MLKDGQRET	MLKDGQRET	MLKDGQRET	MLKDGQRET

Human adult tissues

Cell lines
derived from human cancer



【Figure 6】

Reference No. = C2-906

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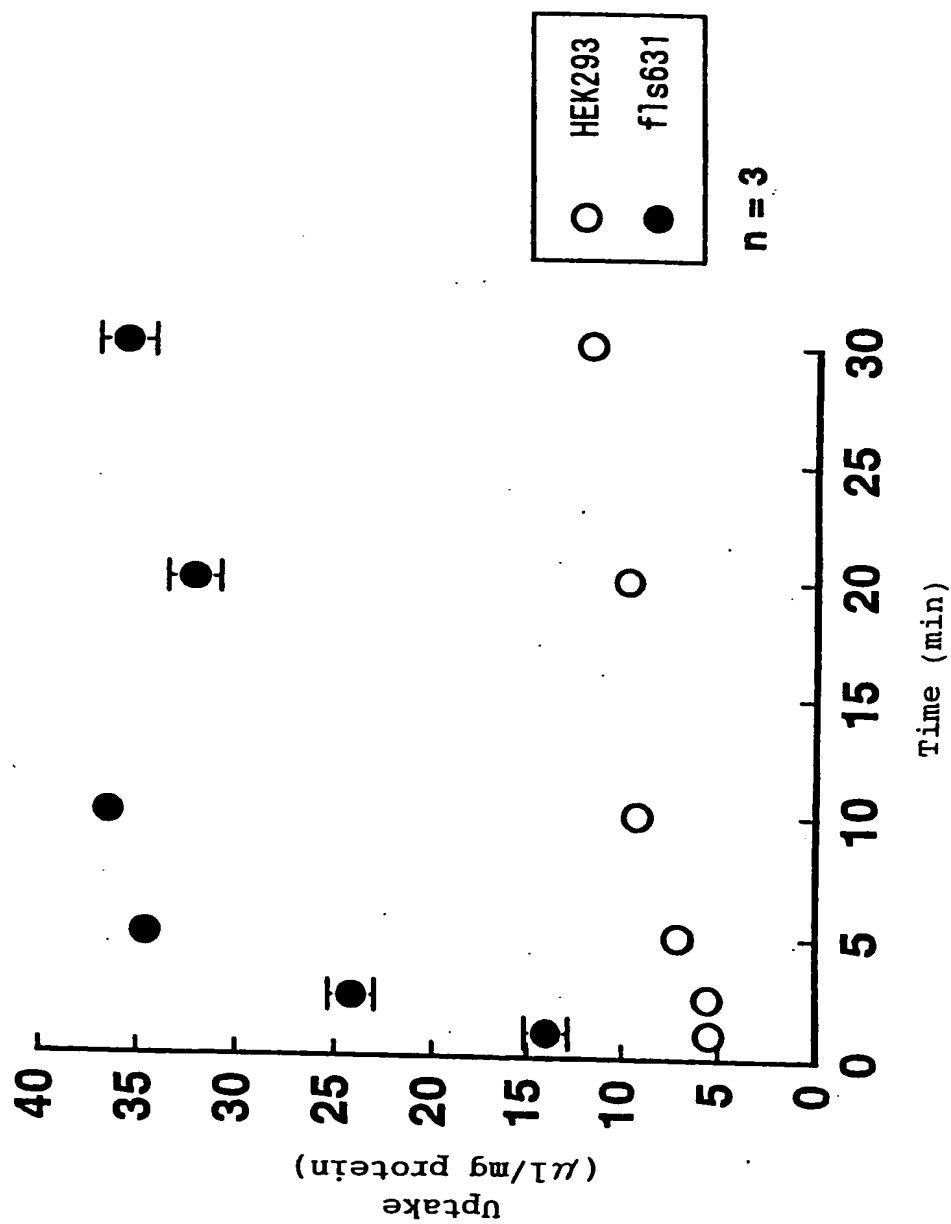
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C	/2
a	/4
n	/2
e	/7
d	/0
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C	/2
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d	/0

11
22
33
44
55
66
77
88
99
aa
bb
cc
dd
ee
ff
gg
hh
ii
jj
kk
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Reference No. = C2-906

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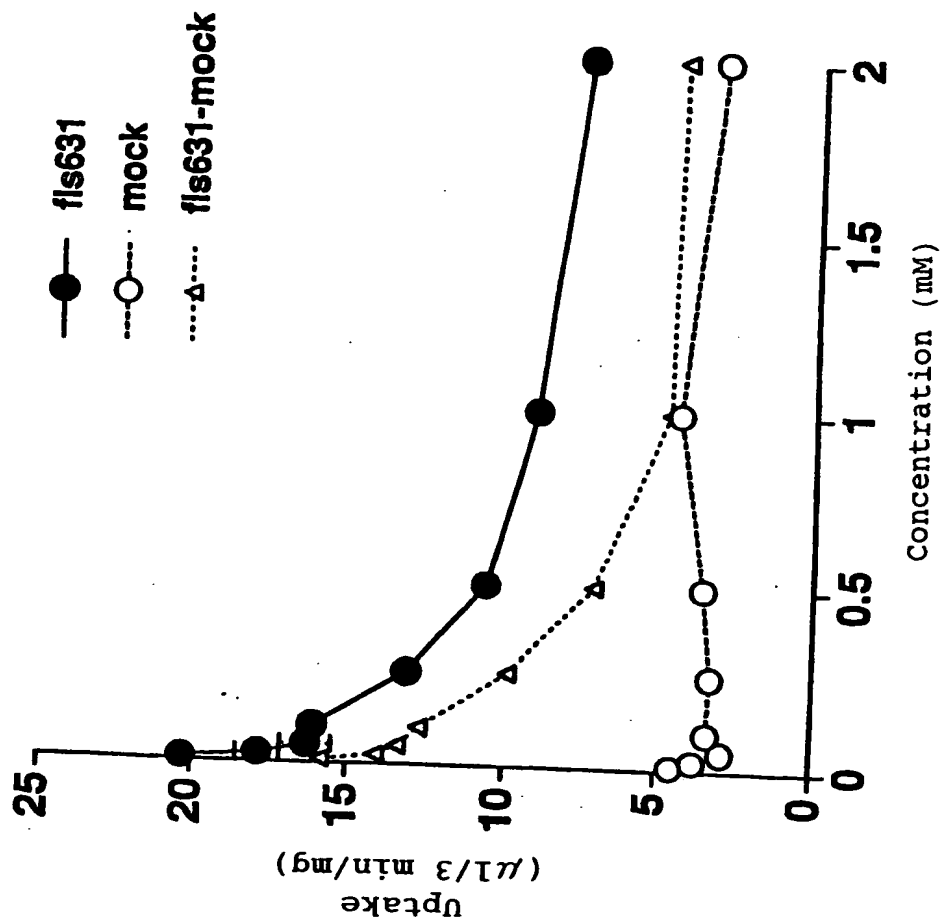
[Figure 7]



Reference No. = C2-906

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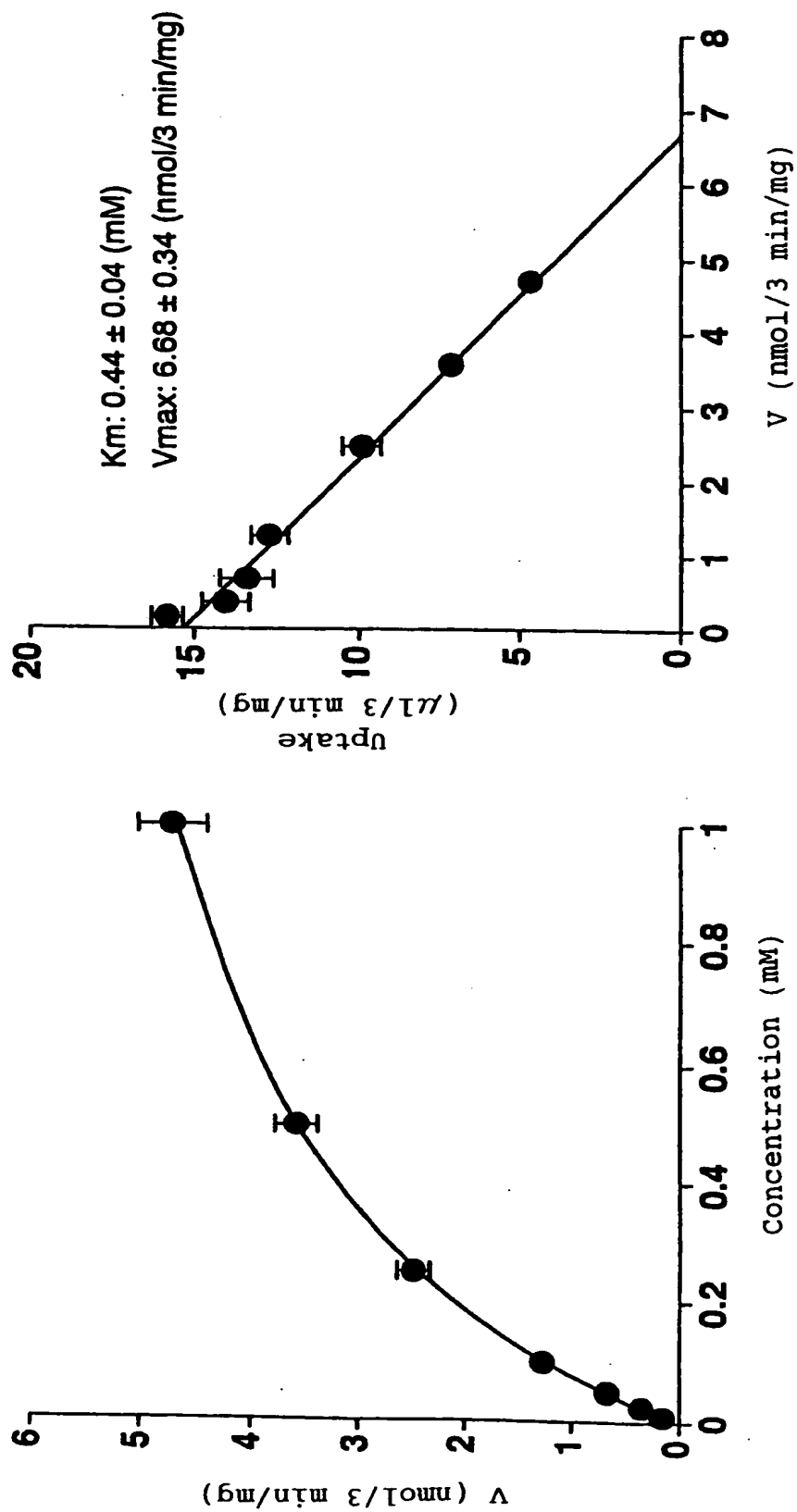
[Figure 8]



Reference No. = C2-906

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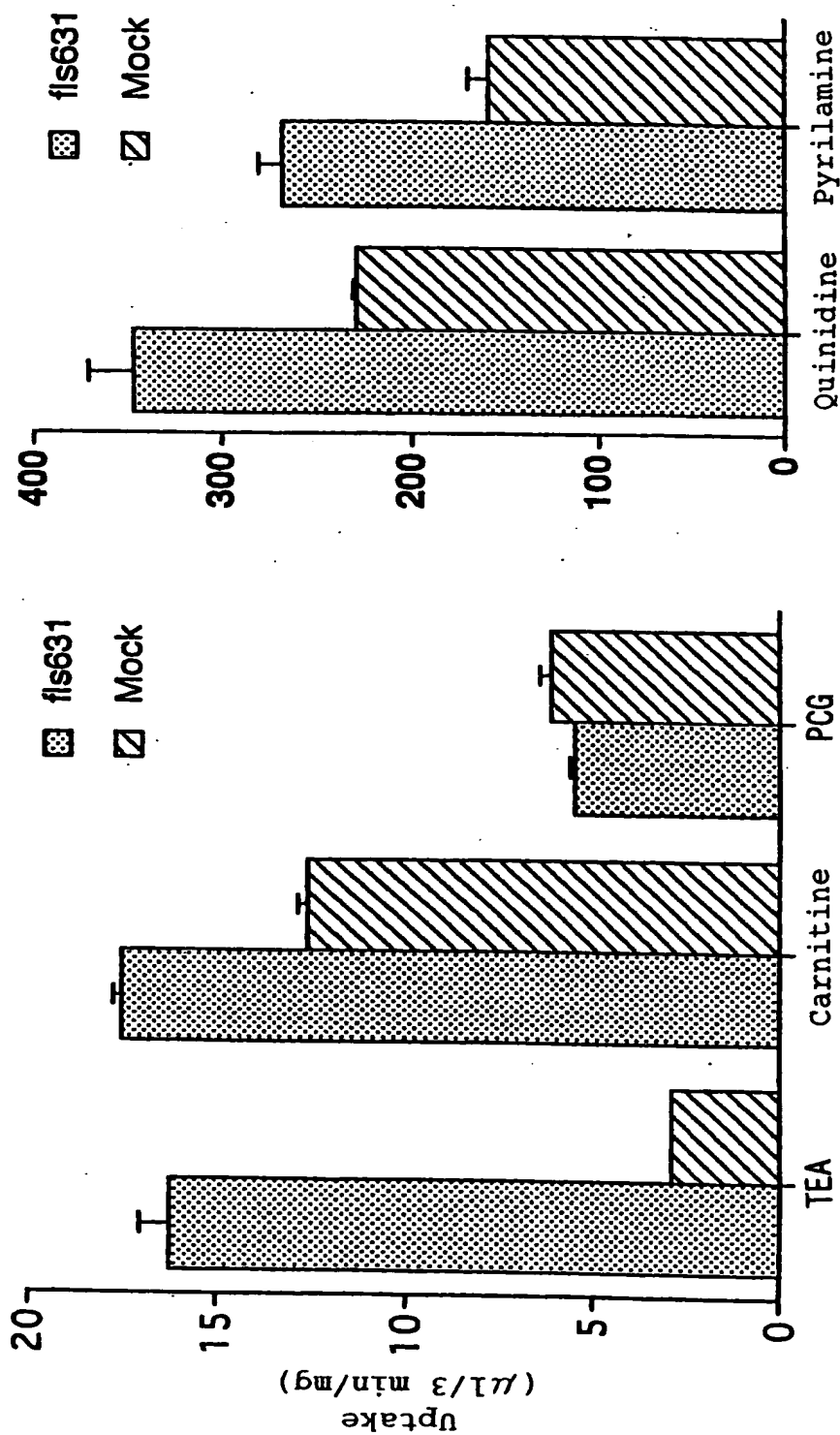
[Figure 9]



Reference No. = C2-906

(10/10)

[Figure 10]



[Document Name] Abstract

[Abstract]

[Problems] The objects are to provide novel transporter genes and their proteins.

5 [Means of Solution] An unknown gene significantly homologous to
known organic cation transporter family proteins has been
successfully isolated by screening a fetal gene library using random
sequencing. The inventors cloned the gene and analyzed its
structure. As a result, it was found that the gene encodes a novel
10 protein which has a high overall homology with organic cation
transporter family proteins. In addition, after studying the
transporter activity of the protein encoded by the isolated gene,
it was revealed that the protein functions as a transporter of
various organic cations.

15 [Selected Drawing] None

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